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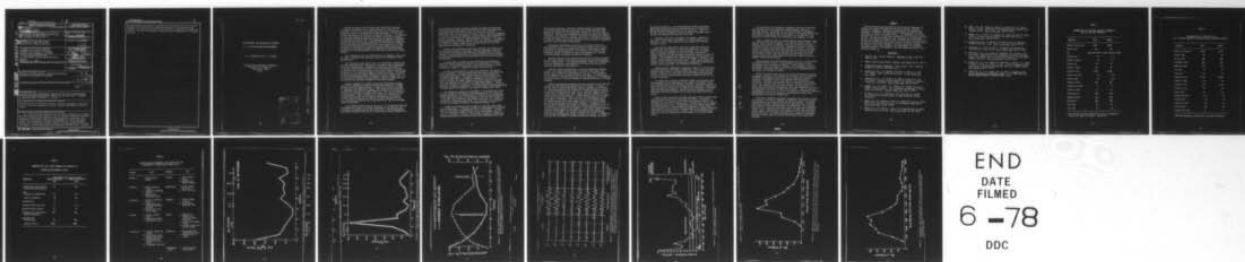
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An overview has been presented as to how dietary allowances for vitamins have been established and utilized in the U.S. Military Nutritional Standards. Research on human vitamin requirements have permitted, in addition, the development of laboratory tests for the assessment of vitamin nutritional status. The procedures are reliable and practical for use in individual evaluations or in military nutrition surveys. Suitable guidelines have been developed for interpreting dietary and biochemical nutrition data. Periodic

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nutrition surveys can be used to determine the nutritional status of military personnel and to evaluate the nutritional adequacy of the food served and consumed. The results can be used as guidance for recommending any indicated corrective actions or adjustments in the nutritional standards of the military services.

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ESTABLISHMENT AND ASSESSMENT OF CERTAIN  
U. S. MILITARY NUTRIENT REQUIREMENTS

H. E. Sauberlich and J. E. Canham

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California 94129  
U.S.A.

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One of the tasks of the Letterman Army Institute of Research is to advise and assist the U.S. military services in matters of nutrition. The services and guidance provided aids in assurance that adequate nutrition is provided the military personnel. Some of the services provided by the Institute include the following: (a) Establish nutrient requirements and develop nutritional and dietary standards for personnel subsisting under normal and special operating conditions (arctic, desert, tropics, etc.); (b) Develop procedures for assessing nutritional status of military personnel; (c) Make periodic surveys of military personnel to determine the nutritional adequacy of the food served and as consumed and to evaluate the nutritional status of the personnel; and (d) Conduct studies on techniques for measuring body composition and provide guidance as to ideal body weight and on weight control.

This presentation will be limited largely to comments on the first two items. Aspects of the remaining items will be included in other presentations.

The Institute and the former unit, U.S. Army Medical Research and Nutrition Laboratory, have been active in studies on the establishment of nutrient requirements. Knowledge of nutritional requirements of military personnel operating under various environmental conditions is essential for the establishment of nutritional standards and for the evaluation of the nutritional adequacy of the food served. Nutritional standards, such as the Medical Services Nutritional Standards (AR 40-25) and the Recommended Dietary Allowances, have two primary uses: (a) to plan diets and rations for individuals or groups of people and (b) to evaluate the nutritional adequacy of the foods consumed.

Dietary standards are opinions based upon experimental studies specifically designed to evaluate nutrient requirements of the human and upon practical experience and information as to nutrient intakes of individuals and population groups. Such judgments must take into consideration that nutrient requirements may differ for individuals as a result of influences of factors such as age, sex, weight, activity, and environment. Since information is limited as to the degree of influence such factors have on nutrient requirements, the various dietary standards provide an allowance for these uncertainties (1). Thus, the dietary allowances are established higher than the estimated average requirement.

In an affluent country such as the U.S.A., it is generally accepted and assumed that errors in nutrient allowances on the high side are preferable to underestimates on the actual need. Such considerations have been incorporated into the U.S. Military Nutritional Standards (2).

These standards "reflect existing knowledge on amounts of nutrients sufficient to maintain adequate nutrition of military personnel under normal conditions, and allow a margin of safety for individual variations" (2). As may be noted in Tables 1 and 2, nutrient allowances for the U.S. military personnel may exceed somewhat those recommended by the U.S. National Academy of Sciences-National Research Council (3). Calories, protein, vitamin C, and riboflavin are examples. The Japanese Recommended Dietary Allowance (Table 3) and the Canadian Recommended Daily Nutrient Intakes (Table 4) for the age 18 year old civilian population are presented for comparison.

Vitamins are recognized as nutrients essential to performing the physiological processes of the body. However, endogenous means are not available to produce them, therefore, exogenous sources must be provided. The requirements for the vitamins appear to be the results of their excretory losses in the urine and feces and through metabolic degradations.

As noted above, personnel at this Institute have conducted a number of studies designed to establish the requirement of various nutrients for the normal adult male. The nutrients studied include vitamin B-6, vitamin C, vitamin A, thiamin, and riboflavin.

Vitamin B-6 (pyridoxine) is used as an example of the type of studies conducted. The quantitative adult male human requirement for this vitamin was established by placing adult male volunteers on controlled experiments. The subjects received either formula diets or special menus that were devoid or inadequate in vitamin B-6. Table 5 provides the general composition of the liquid formula diets that were employed in the vitamin B-6 requirement studies. Similar diets were utilized in studies on the human requirement for vitamin C, vitamin A, thiamin and riboflavin. The diets permit controlled depletion and repletion of the subjects in the specific vitamin under study.

For studies on vitamin B-6 requirement, a low protein and a high protein diet were used because of the association of the vitamin to protein metabolism. The requirement for vitamin B-6 can be evaluated in the human on the basis of (a) changes in urine or serum levels of vitamin B-6; (b) erythrocyte transaminase activities; (c) response to tryptophan or methionine load tests; (d) 4-pyridoxic acid excretion; and (e) the presence of abnormal electroencephalograms (4-6).

Vitamin B-6 deficiency can be induced in the adult male within two or three weeks. In isotopic studies, we demonstrated that the body pool of vitamin B-6 is approximately 22 to 27 milligrams. The half-life of isotopically labeled pyridoxine was approximately 15 to 20 days (7). As noted in Figure 1, the urinary excretion of vitamin B-6 dropped dramatically and increased again when supplementation of the

vitamin was instituted. Similarly, abnormal amounts of xanthurenic acid were excreted following tryptophan loads within two weeks after placing the subjects on the high-protein vitamin B-6 deficient diet (Figure 2). Vitamin B-6 supplementation promptly corrected the abnormal xanthurenic acid excretion. Subjects receiving the low-protein vitamin B-6 deficient diet did not reach the same degree of pyridoxine deficiency as based on urinary excretion of xanthurenic acid following a tryptophan load until the sixth week of deficiency.

Erythrocyte transaminase activities also reflect vitamin B-6 status (Figure 3) (5). This was particularly evident in the *in vitro* stimulation of erythrocyte glutamic oxaloacetic transaminase (EGOT) activity by pyridoxal phosphate. Thus, EGOT activity declines with inadequate intakes of vitamin B-6 and is accompanied by an increase in the percentage stimulation by pyridoxal phosphate.

The most marked clinical manifestation observed during the vitamin B-6 deficiency period in the volunteer subjects were electroencephalographic abnormalities. Electroencephalographic changes are indicated in Figure 4. Repletion of the subjects with vitamin B-6 corrected the electroencephalographic abnormalities.

From the results obtained from a series of controlled volunteer studies, it has been concluded that for the adult male human, the optimal daily vitamin B-6 requirement was 1.75 to 2.0 milligrams per day for subjects with a protein intake of 100 grams. An allowance of 2.0 milligrams of vitamin B-6 per day has been adopted as the recommended allowance by the U.S. National Academy of Sciences and the U.S. Military Services.

Similar experimental approaches have been utilized at the Institute to establish the adult human requirement for vitamin C. In these studies, adults received controlled intakes of ascorbic acid (vitamin C). Their body pools of the vitamin were followed through the use of isotopically labeled ascorbic acid (8-11). When the subjects received a diet free of ascorbic acid, their plasma and whole blood levels of vitamin C fell rapidly (Figure 5). Plasma ascorbic acid fell to levels lower than those of whole blood. Ascorbic acid disappeared from the urine early in depletion. The first signs of scurvy appeared when the plasma ascorbic acid levels ranged from 0.13 to 0.24 milligrams per 100 ml and the pool size had been depleted to a range of 96 to 490 mg from an average initial pool of 1,500 mg. Supplements of 10 mg of ascorbic acid per day were sufficient to alleviate and cure the clinical signs of scurvy in the subjects. The isotopic labeling studies permitted a measurement of the actual utilization and turnover of vitamin C in the body. The results indicated that the adult human male utilizes approximately 30 mg of ascorbic acid daily. An intake of 45 mg per day of ascorbic acid would maintain an adequate and normal



body pool of vitamin C. The recent National Academy of Sciences Recommended Dietary Allowance has adopted 45 mg of vitamin C per day as the recommended allowance for the adult male. The U.S. Military Services recommend an intake of 60 mg of vitamin C which provides an allowance for stressful conditions.

The adult human male requirements for thiamin, riboflavin, and vitamin A have been investigated in a similar manner at our Institute. Some of these studies have been published in detail elsewhere (12-15).

Once nutrient requirements have been established, practical procedures are then necessary to determine whether or not these needs are being provided by the diets consumed. The nutritional status of military personnel in the U.S.A. and in over thirty foreign countries has been evaluated through the conduct of nutrition surveys. The assessments when conducted periodically can monitor changes in food usage that may result from changes in economics, food supply, feeding systems, and other factors. Such surveys generally include clinical and dental examinations, anthropometric measurements, dietary intake evaluations, and biochemical assessments.

Dietary survey information is generally subject to less accuracy than that obtained by biochemical procedures. Errors are involved in estimating the amount of different foods eaten and of their nutrient content. Moreover, an individual's pattern of food consumption may not necessarily be characterized by the diet consumed on any one day or even in one week.

In recent years, various biochemical methods and techniques have been developed for the evaluation of the vitamin nutritional status of population groups or of individual subjects. Biochemical measurements represent an objective assessment of the nutritional status of an individual and may provide pre- or subclinical information. Depending upon the measurement employed, information may be obtained as to an individual's present or recent and sometimes long-range nutritional status.

Many of these procedures have been developed at this Institute and utilized extensively in military nutrition surveys. Biochemical techniques are available for assessing the nutritional status of individuals for most of the vitamins (15). The techniques can in general be categorized as follows: (a) measurement of the vitamin under study in urine and blood; (2) measurement of a metabolite of the vitamin under study in urine or blood; (3) measurement of an abnormal metabolite resulting from a deficiency of a vitamin; (4) perform functional or load tests; and (5) measurement of the product of the vitamin under study.



The validity of the biochemical techniques have been established mainly through the use of controlled human volunteer studies as described earlier. Guidelines for the interpretation of biochemical data have also been established as the result of such investigation (15). This permits an evaluation and classification of an individual as to whether his nutrient intakes are inadequate, low, adequate, or high.

Table 6 summarizes some of the more commonly used laboratory tests that are useful for the assessment of vitamin nutritional status. For example, serum vitamin A (retinol) levels can be used to assess the nutritional status of this nutrient. Prolonged low dietary intakes of vitamin A correlate with serum concentrations of retinol. Since the vitamin is stored in the liver, low serum levels of vitamin A reflect not only low intakes of the nutrient but also depleted liver stores. Serum vitamin A levels above 30  $\mu\text{g}/100\text{ ml}$  indicate liver stores of vitamin A while serum values below this generally indicate low or inadequate intakes of the vitamin.

Serum ascorbic acid concentrations can be used in a similar manner to evaluate vitamin C status. Serum ascorbic acid shows a linear relationship with the intake of vitamin C. Serum ascorbic acid concentrations below 0.20  $\text{mg}/100\text{ ml}$  indicate low or inadequate intakes of the vitamin. Continued serum values of less than 0.10  $\text{mg}/100\text{ ml}$  will invariably lead to signs of scurvy.

Urinary excretion levels can be used in the nutritional assessment of thiamin, riboflavin, and vitamin B-6. Clinical signs of thiamin or riboflavin deficiency may occur when urinary thiamin or riboflavin excretion, respectively, fall in the range of 20 to 30  $\mu\text{g}/\text{gm}$  of creatinine. Similarly, urinary excretion of vitamin B-6 falls below 20  $\mu\text{g}/\text{gm}$  of creatinine when clinical signs of a vitamin B-6 deficiency occur. More recently, sensitive and reliable functional tests, representing enzyme activity measurements, have been introduced for use in the nutritional assessment of these three vitamins. Thus, erythrocyte transketolase assays are used for thiamin, erythrocyte glutathione reductase measurements are used for riboflavin, and erythrocyte transaminase determinations are used for vitamin B-6.

When these biochemical assessment techniques are utilized in nutrition surveys, what type of findings are obtained? With well-nourished normal persons, low or deficient values are not observed. In random populations, however, subjects with values indicating poor nutrition with respect to one or more nutrients are encountered. As an example, Figures 6 and 7 represent distribution plots for serum and erythrocyte folacin levels. Serum folacin levels below 3.0  $\text{ng}/\text{ml}$  and erythrocyte folacin levels below 160  $\text{ng}/\text{ml}$  are indicative of less than acceptable folacin nutriture. The figures indicate that a number of individuals with poor dietary intakes of folacin were present in the population examined.

### SUMMARY

An overview has been presented as to how dietary allowances for vitamins have been established and utilized in the U.S. Military Nutritional Standards. Research on human vitamin requirements have permitted, in addition, the development of laboratory tests for the assessment of vitamin nutritional status. The procedures are reliable and practical for use in individual evaluations or in military nutrition surveys. Suitable guidelines have been developed for interpreting dietary and biochemical nutrition data. Periodic nutrition surveys can be used to determine the nutritional status of military personnel and to evaluate the nutritional adequacy of the food served and consumed. The results can be used as guidance for recommending any indicated corrective actions or adjustments in the nutritional standards of the military services.

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TABLE 1

RECOMMENDED DAILY DIETARY NUTRIENT ALLOWANCES  
FOR U.S. MILITARY PERSONNEL\*

NUTRIENT	MEN	WOMEN
Calories (Kcalories)	3,400	2,400
Protein (gm)	100	80
Fat	Less than 40% of Total Calorie Intake	
Thiamin (mg)	1.7	1.2
Riboflavin (mg)	2.0	1.7
Niacin (mg)	22	16
Vitamin C (mg)	60	60
Vitamin A (IU)	3,000	5,000
Vitamin B-6 (mg)	2.0	2.0
Folacin (mg)	0.4	0.4
Vitamin D (IU)	400	400
Vitamin B-12 ( $\mu$ g)	3	3
Vitamin E (IU)	15	12
Calcium (mg)	800	800
Magnesium (mg)	350	300
Phosphorus	800	800
Zinc (mg)	15	15
Iron (mg)	14	18

\*For military personnel moderately active in a temperate climate; for ages 17-25 years. (AR 40-25)



TABLE 2

RECOMMENDED DAILY DIETARY OF THE  
U.S. NATIONAL ACADEMY OF SCIENCES-NATIONAL RESEARCH COUNCIL\*

NUTRIENT	MEN	WOMEN
Calories (Kcalories)	3,000	2,100
Protein (gm)	54	46
Calcium (mg)	800	800
Phosphorus (mg)	800	800
Magnesium (mg)	350	300
Zinc (mg)	15	15
Iron (mg)	10	18
Vitamin A (IU)	5,000	4,000
Vitamin C (mg)	45	45
Vitamin D (IU)	400	400
Vitamin E (IU)	15	12
Niacin (mg)	20	14
Vitamin B-6 (mg)	2.0	2.0
Thiamin (mg)	1.5	1.1
Riboflavin (mg)	1.8	1.4
Folacin (mg)	0.4	0.4
Vitamin B-12 ( $\mu$ g)	3	3

\*NAS-NRC Allowances, Revised 1974; for ages 19-22 years.

TABLE 3

JAPANESE RECOMMENDED DIETARY ALLOWANCES  
(1975-1980: Ministry of Health & Welfare)

NUTRIENT	MALE*	FEMALE*
Energy (Kcalories)**	2,700	2,100
Protein (gm)	80	65
Calcium (mg)	700	600
Iron	12	12
Vitamin A (IU)	2,000	1,800
Thiamin (mg)	1.1	0.8
Riboflavin (mg)	1.4	1.1
Niacin (mg)	18	14
Vitamin C (mg)	50	50
Vitamin D (IU)	100	100

\*For age 18 years.

\*\*Moderate activity.

TABLE 4

RECOMMENDED DAILY NUTRIENT INTAKES: CANADA  
(Bureau of Nutritional Sciences, Health & Welfare of Canada:1974)

NUTRIENT	MALE*	WOMEN*
Energy (Kcalories)	3,200	2,100
Protein (gm)	54	43
Calcium (mg)	1,000	700
Phosphorus (mg)	1,000	700
Magnesium (mg)	300	250
Iron (mg)	14	14
Zinc (mg)	12	11
Vitamin A ( $\mu$ gRE)**	1,000	800
Vitamin D ( $\mu$ g)***	2.5	2.5
Vitamin E (mg)	10	6
Vitamin C (mg)	30	30
Thiamin (mg)	1.6	1.1
Niacin (mg)	21	14
Riboflavin (mg)	2.0	1.3
Vitamin B-6 (mg)	2.0	1.5
Folacin (mg)****	0.2	0.2
Vitamin B-12 ( $\mu$ g)	3	3

\*For age group 16-18 years.

\*\*1  $\mu$ g retinol equivalent corresponds to a biological activity in humans equal to 1  $\mu$ g retinol (3.33 IU) and 6  $\mu$ g  $\beta$ -carotene (10 IU).

\*\*\*As cholecalciferol (1  $\mu$ g = 40 IU vitamin D activity)

\*\*\*\*As "free folate."

TABLE 5

COMPOSITION OF THE LIQUID FORMULA DIET EMPLOYED IN  
VITAMIN B<sub>6</sub> REQUIREMENT STUDIES

INGREDIENT	Consumption per subject per day	
	Low-protein diet (gm)	High-protein diet (gm)
Vitamin-free casein (micro-pulverized; 92% protein)	33	110
Fats		
Coconut oil (saturated)	84	84
Corn oil (Mazola)	36	36
DL-Methionine	2	--
Vitamin mixture (B <sub>6</sub> -free)	10	10
Special mineral mixture	13	13
Carbohydrates (cornstarch, dextrin, glucose)	390	390
Diet provided		
Protein (gm)	30	100
Calories (kcal)	2800	2800



TABLE 6

FUNCTIONAL AND BIOCHEMICAL TESTS USEFUL FOR THE  
ASSESSMENT OF VITAMIN NUTRITIONAL STATUS

VITAMIN	TEST	VITAMIN	TEST
Vitamin A	1. Serum retinol levels	Folacin	1. Serum folacin levels 2. Erythrocyte folacin levels
Vitamin C	1. Serum ascorbate levels 2. Leukocyte ascorbate levels 3. Whole blood ascorbate levels	Vitamin B <sub>12</sub>	1. Serum vitamin B <sub>12</sub> levels 2. Schilling test
Riboflavin	1. Urinary riboflavin level 2. Erythrocyte glutathione reductase activity 3. Erythrocyte riboflavin levels	Vitamin E	1. Serum vitamin E levels 2. Erythrocyte hemolysis test
Thiamin	1. Urinary thiamin level 2. Erythrocyte transketolase activity	Niacin	1. Urinary N'-Methyl nicotinamide levels (N'-Me N) 2. Urinary 2-pyridone levels 3. Urinary 2-pyridone/N'-Me N ratio
Vitamin B <sub>6</sub>	1. Urinary vitamin B <sub>6</sub> levels 2. Erythrocyte transaminase activities 3. Erythrocyte and serum vitamin B <sub>6</sub> levels 4. Tryptophan load test	Vitamin D	1. Serum alkaline phosphatase levels 2. Serum Ca and P levels
		Pantothenic Acid	1. Urinary excretion levels

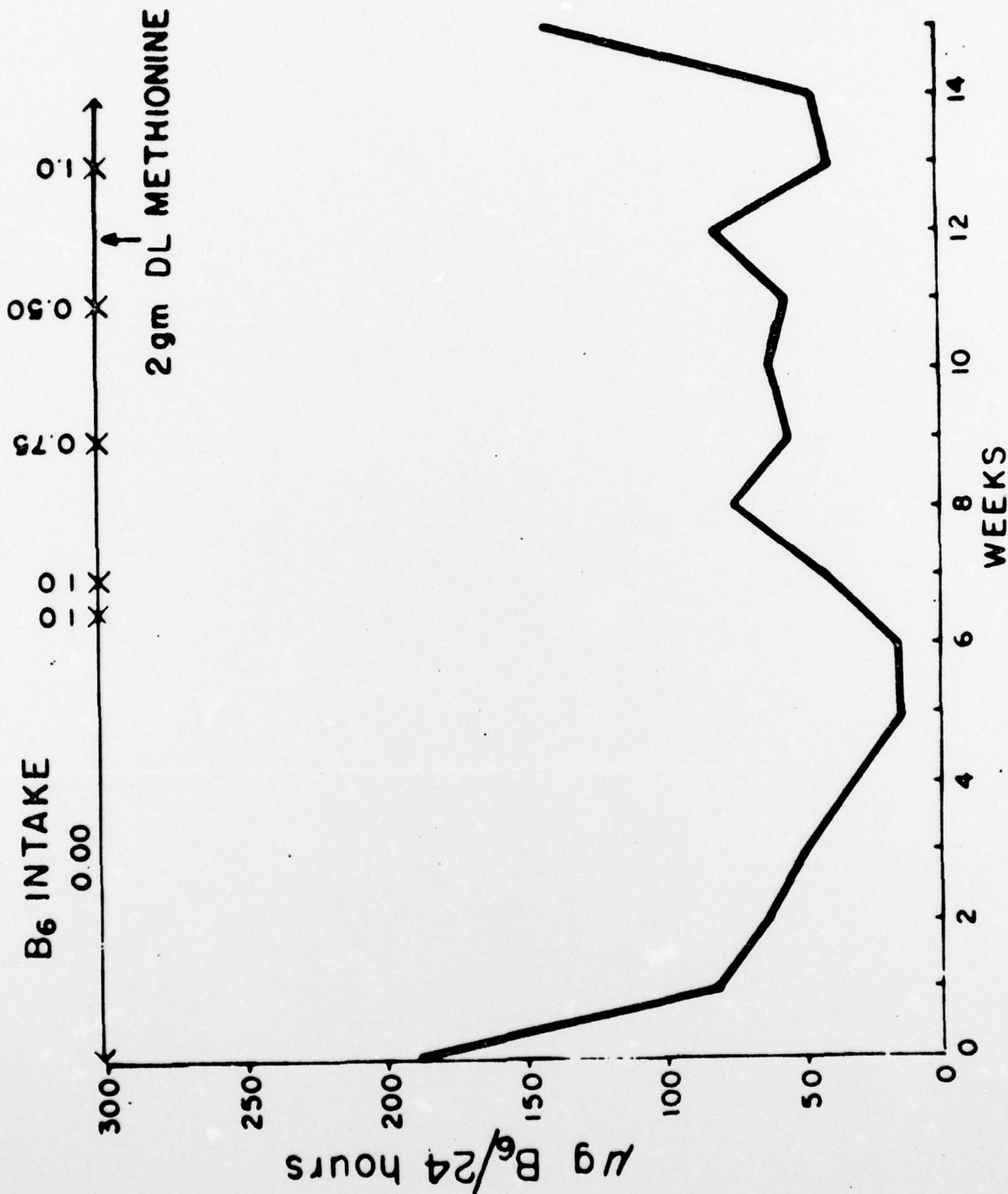


Figure 1. Effect of vitamin B-6 intake on the urinary excretion of vitamin B-6 by

**B<sub>6</sub> INTAKE**

**WEEKS**

**ug B<sub>6</sub>/24 hours**

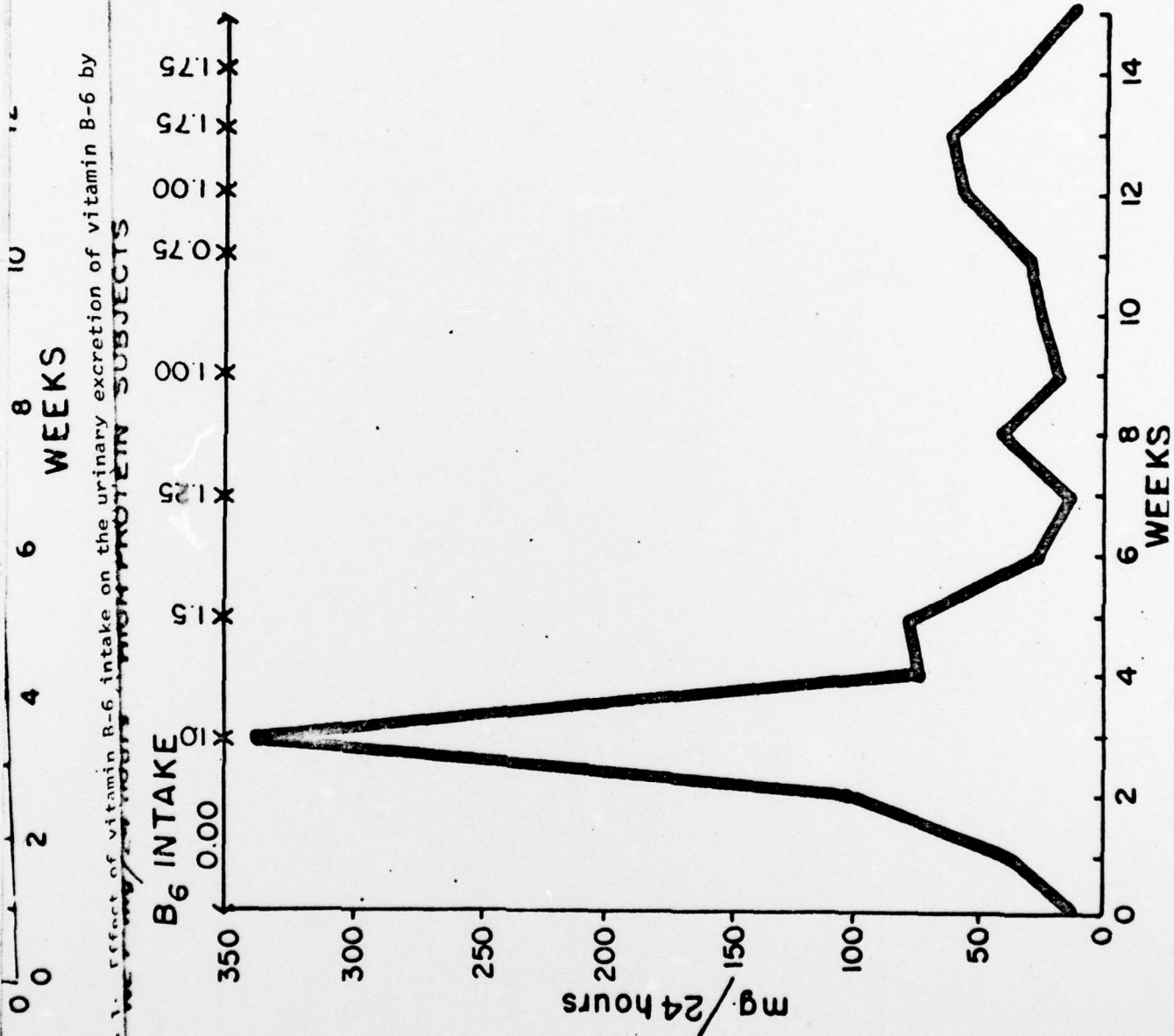


Figure 2: Effect of vitamin B-6 intake on the urinary excretion of xanthurenic acid by the adult male human following a 10 gm DL-tryptophan load.

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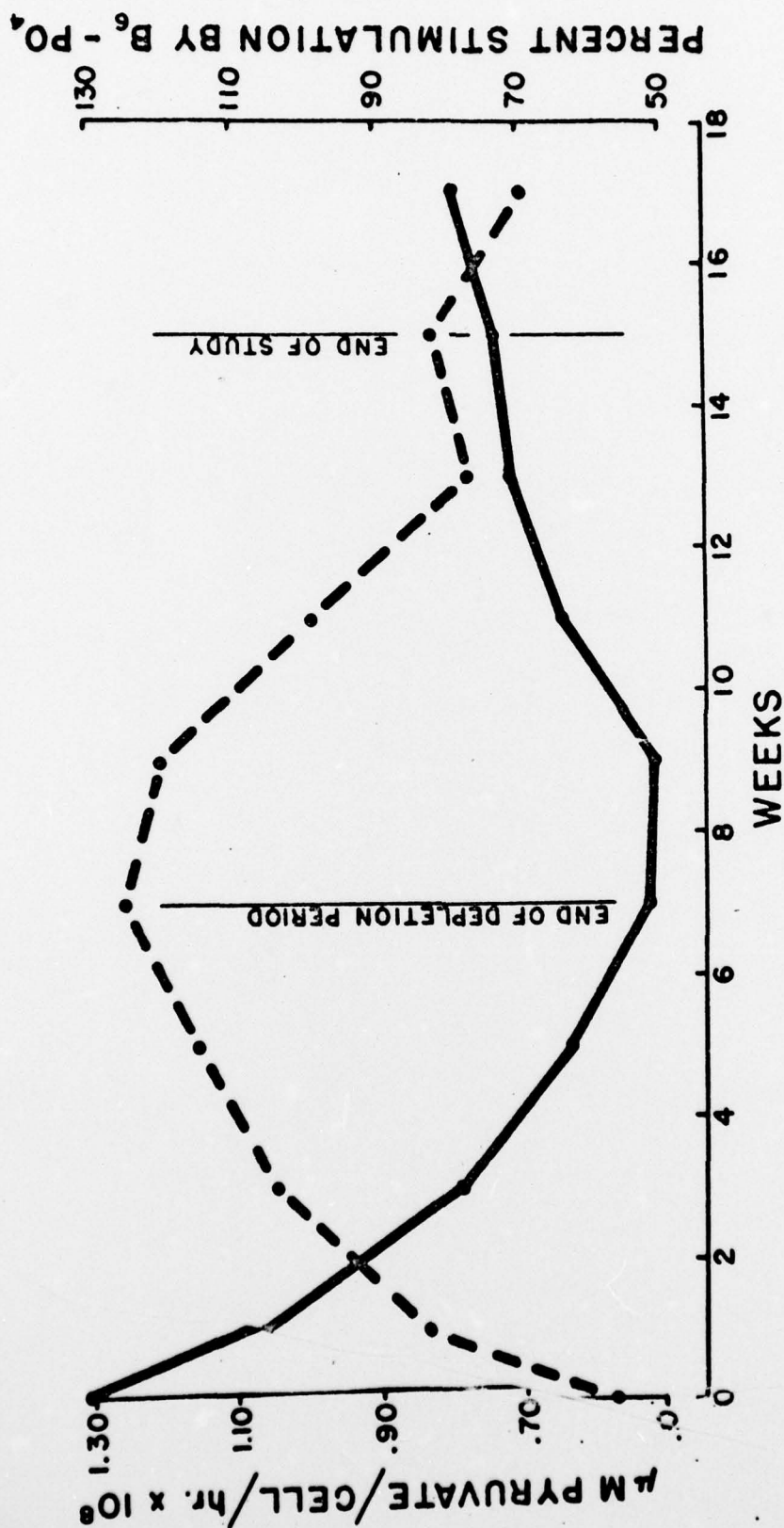


Figure 3: Glutamic oxaloacetic transaminase activity in adult male human erythrocytes and per cent in vitro pyridoxal phosphate stimulation during vitamin B-6 depletion and repletion (low protein diet).

3-SECOND INTERVALS

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20 SEPT.

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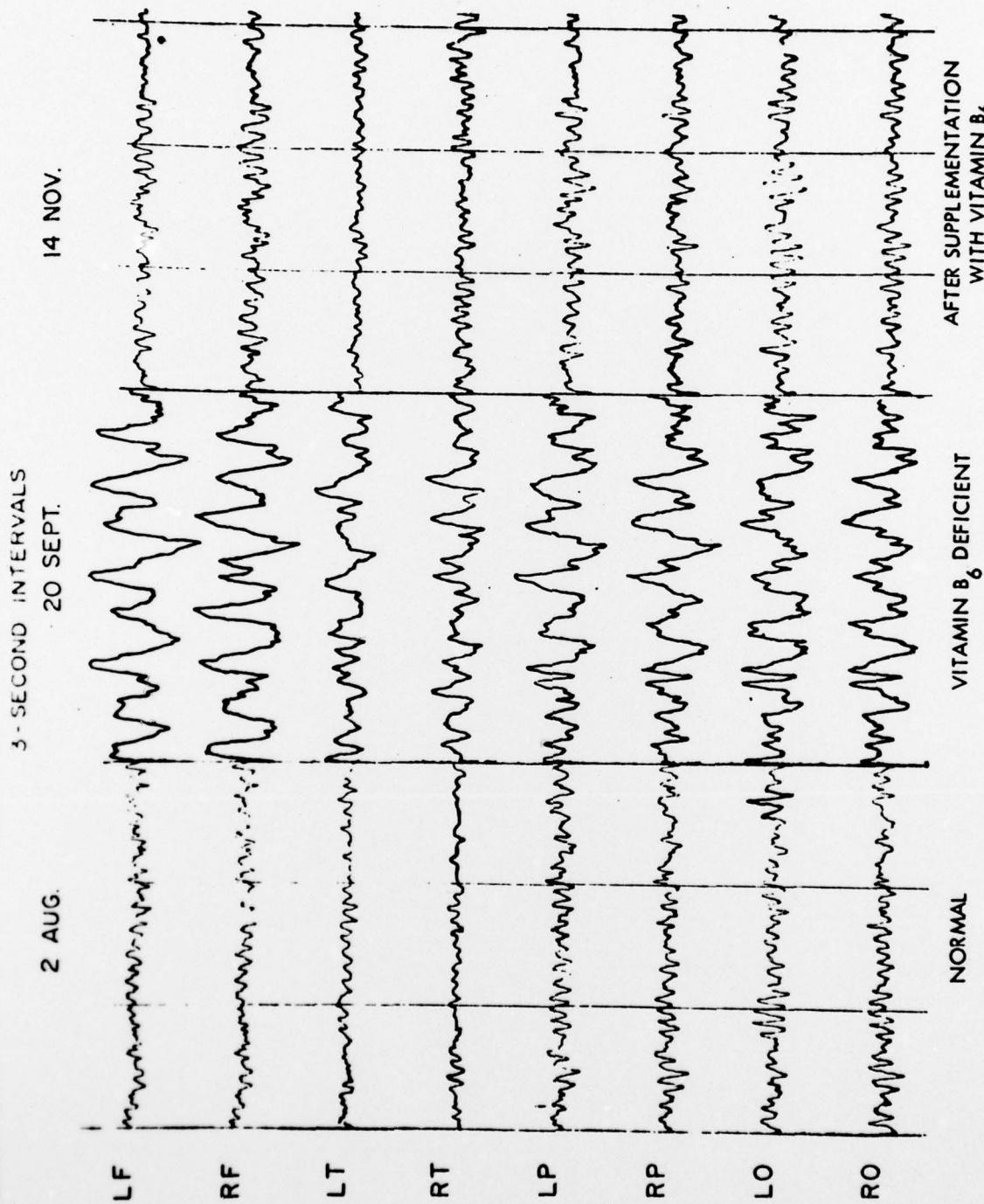


Figure 4: Electroencephalogram of an adult male human on a vitamin B-6 deficient low protein diet. (Left: prior to deficiency; Center: a peak vitamin B-6 deficiency, with abnormal slowing and increased amplitude of the waves at all leads; Right: after vitamin B-6 repletion, providing a normal pattern)

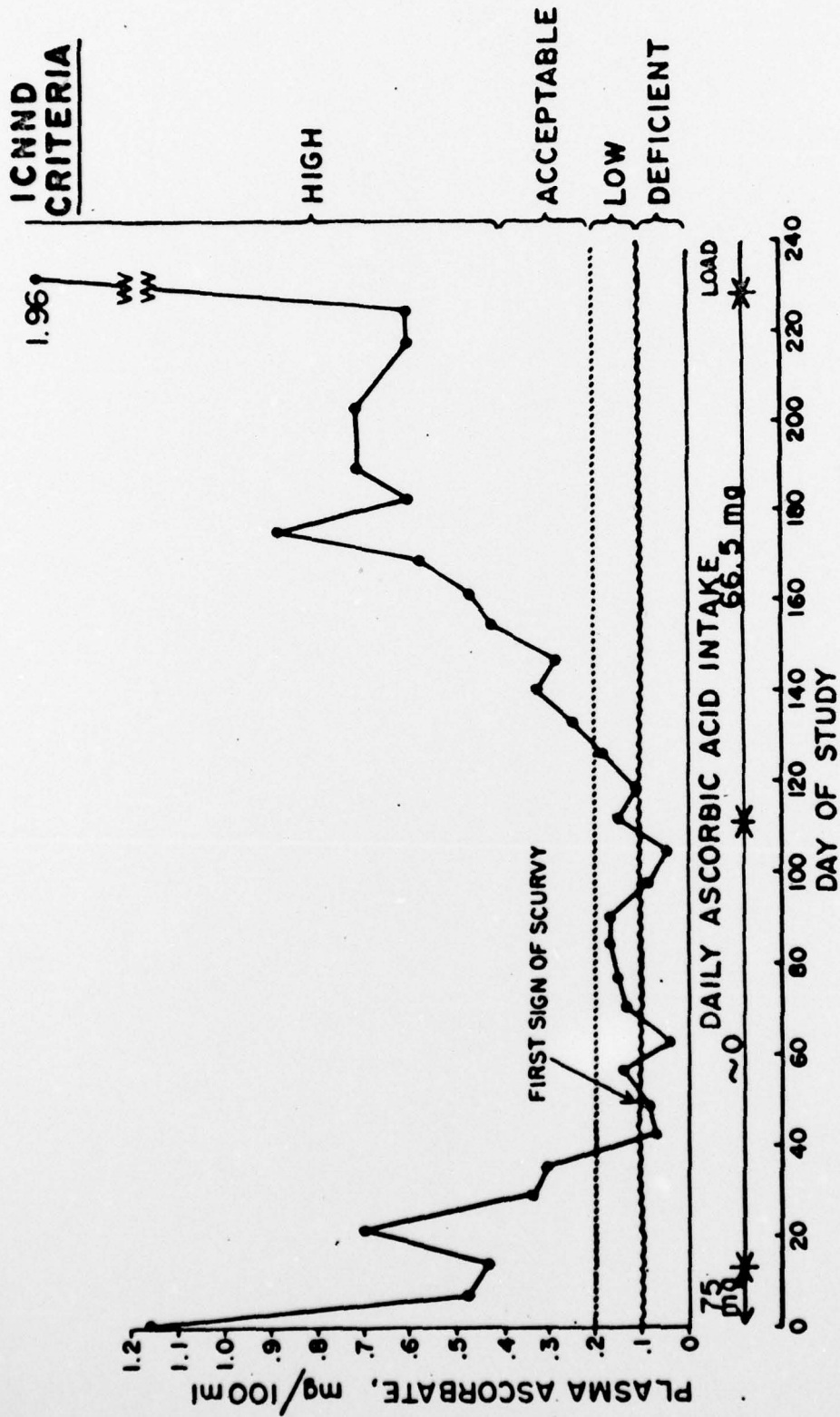


Figure 5: Effect of ascorbic acid (vitamin C) intake on the plasma ascorbic acid levels in the adult male human.

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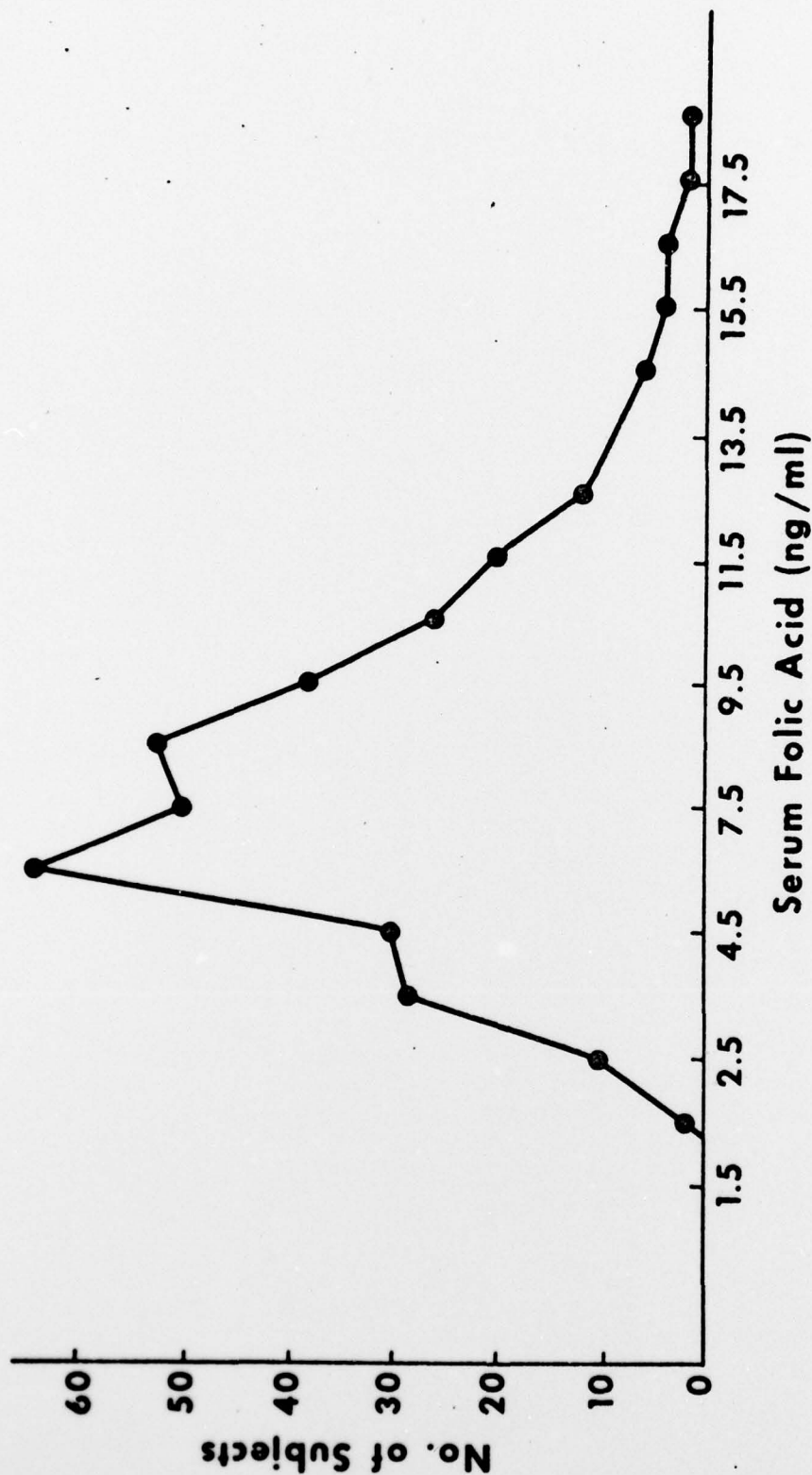


Figure 6: Distribution plot of serum folic acid values for 348 females age 18-28 years. Mean serum folic acid value was  $8.2 \pm 2.8$  ng/ml. Range 2.5-18.0 ng/ml. One subject had a value of less than 3.0 ng/ml, while 66 had values less than 6.0 ng/ml.



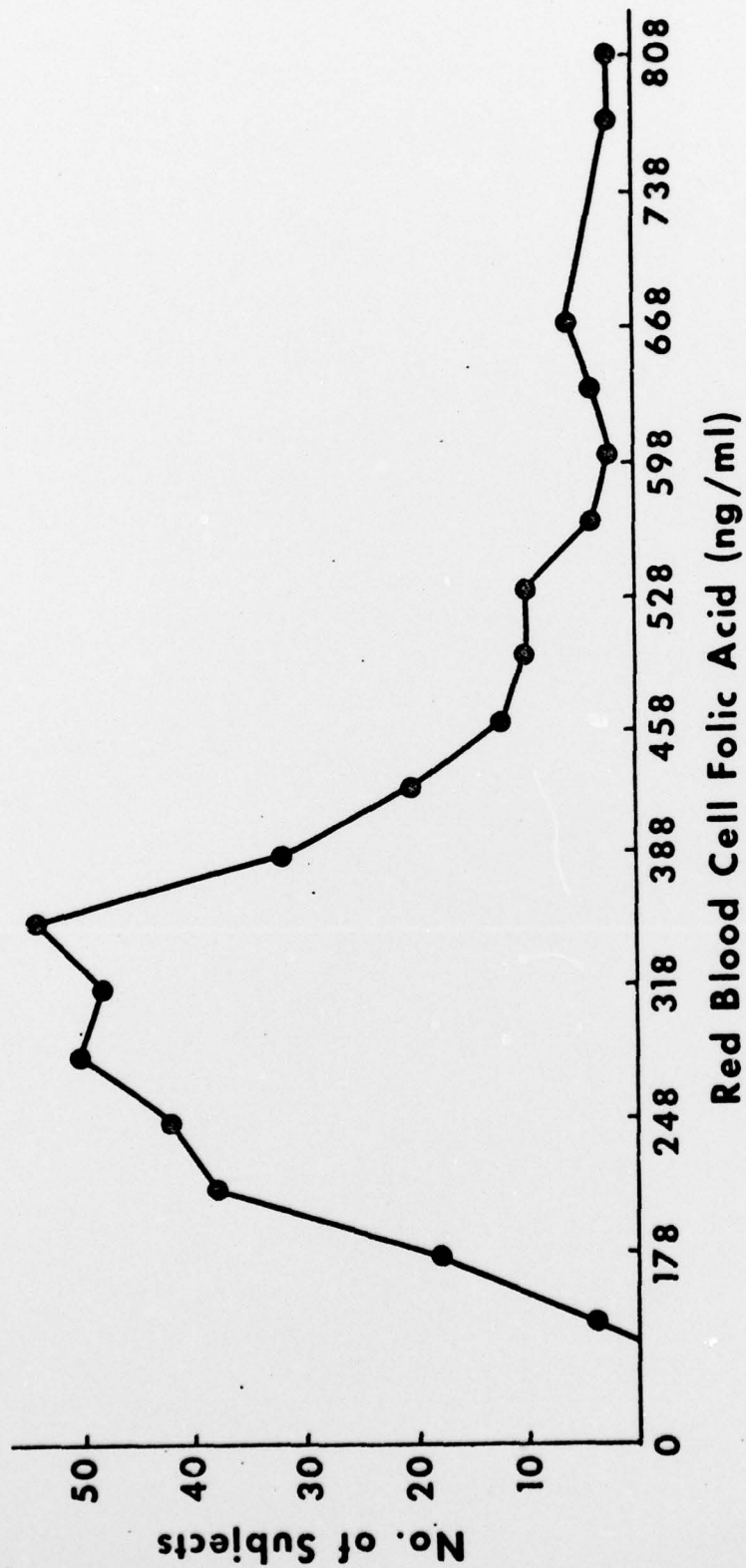


Figure 7: Distribution plot of red blood cell folic acid values for 347 females age 18-28 years. Mean red blood cell folic acid value was  $330 \pm 108$  ng/ml. Range 125-825 ng/ml.